Cylic Thioester-Containing Macromonomers for Cross-Linking of Hydrogels by Native Chemical Ligation

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Statement of Purpose: Crosslinking and solidification of hydrogels under physiological conditions is desired for many medical applications such as drug delivery, tissue engineering and tissue repair, yet identification of chemoselective and biocompatible cross-linking methods is still challenging. We previously reported on the use of native chemical ligation (NCL) reaction[1] for *in situ* cross-linking of macromonomers into hydrogels.[2,3] The NCL reaction occurs under mild conditions between N-terminal cysteine (Cys) and thioester conjugated macromonomers, however release of a thiol compound occurs during the reaction which can have adverse biological effects. In this work we developed a novel cyclic thioester conjugated macromonomer for in NCL hydrogel formation. The use of cyclic thioester-containing macromonomers dramatically decreased toxicity of the hydrogel system during interaction with cells, as compared to hydrogels formed using linear thioester-conjugated molecules that release free thiol molecules during NCL crosslinking. This catalyst-free and side-product free crosslinking method by NCL reaction using cyclic thioesters to form hydrogels will lead to greater pharmaceutical and medical use of such hydrogel materials.

Methods: 4-armed poly(ethylene glycol) (PEG) macromonomers terminated with N-terminal cysteine (4A-PEG-Cys), C-terminal cyclic thioester (4A-PEG-CThE) or C-terminal linear thioester (4A-PEG-LThE) were manually synthesized. Maleimide-terminated peptide MA-GRGDSPG-NH_2 (MA-RGD) was synthesized as reported previously.[2] To form hydrogels presenting cell-adhesive peptides for cell culture, maleimide-terminated peptide were added to the solution of PEG-Cys at a molar ratio of 1:25 (MA-RGD: PEG-Cys).[3] Subsequent mixing of 30 µl PEG-Cys and 30 µl of PEG-CThE or PEG-LThE in DMEM (pH 7.6) at 37°C for 2 hours resulted in hydrogel disks. Cultured mouse islet derived MIN-6 cells were collected and resuspended in DMEM media containing 10% FBS. 100 µl of cell suspension was deposited on top of the hydrogel samples and cultured for 24 hours. Viability of the cells cultured on top of hydrogels was imaged using calcein AM to detect live cells and euthidium homodimer-1 to detect dead cells.

Results: The novel cyclic thioester modified PEG macromonomer for native chemical ligation is shown in Figure 1. Reaction between the cyclic thioester and cysteine results in formation of a native peptide bond without the release of free thiol side-products as in conventional native chemical ligation where linear thioesters are used.[2] 4A-PEG-Cys can crosslink efficiently with both 4A-PEG-LThE and 4A-PEG-CThE to form hydrogels *in situ* in a physiologically relevant range of pH 7—8.5. The use of cyclic thioester resulted in RGD-presenting hydrogel where MIN6 cells remained viable whereas on the NCL hydrogel formed using linear thioester cells showed much reduced survival, as a result from their exposure to high concentration of free thiols released to the surrounding media.[4]

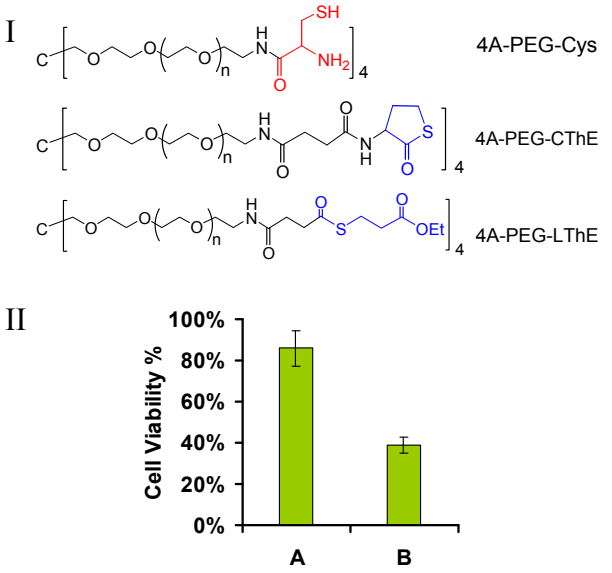

Figure 1. (I) Structures of 4A-PEG-CThE and 4A-PEG-LThE, which form hydrogels through NCL reaction with 4A-PEG-Cys. (II) MIN6 cells cultured on surface of hydrogel crosslinked using 4A-PEG-CThE (A) had significantly improved viability compared to cells cultured on hydrogel formed using 4A-PEG-LThE (B).

Conclusions: We reported a novel strategy for hydrogel crosslinking by native chemical ligation between cyclic-thioester and cysteine-containing macromonomers. Compared to linear thioester, the use of cyclic thioester in NCL crosslinking of hydrogels eliminates cytotoxic thiol side product release, improving the biocompatibility of NCL reaction for constructing injectable hydrogel materials for tissue engineering and other medical applications.

References:

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